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Fluorescent detection of coenzyme A by analyte-induced aggregation of a cationic conjugated polymer

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A new cationic conjugated polymer was designed and synthesized to optically discriminate coenzyme A(CoA) among structurally similar biomolecules, ATP, ADP and AMP. The analyte-induced aggregation of the conjugated polymer by π-stacking between their main chains leads to the fluorescence quenching. Except for the similar adenosine and phosphate moieties as those in ATP, ADP and AMP, the CoA molecule also includes a long side chain that is favorable for hydrophobic interactions. Thus, CoA can form a complex with oppositely charged conjugated polymer by cooperative electrostatic and hydrophobic interactions, whereas ATP, ADP and AMP form the complexes with oppositely charged conjugated polymer mainly by electrostatic interactions. The increase of the ion strength of the assay solution screens the electrostatic attractions, and the remaining hydrophobic interactions dominate the formation of PFP-PTF/CoA complex. At this case, the quenching efficiency of PFP-PTF by CoA is much higher than that by ATP, ADP and AMP, which impart the PFP-PTF to sense CoA from these interferencing species.

conjugated polymers, coenzyme A, biosensor, complex, aggregates, fluorescence quenching

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Since the coenzyme A (CoA) was discoved fifty years ago [1], it has been found to be concerned with many kinds of biochemical reactions [2]. One of the most important functions of CoA is the transfering of acyl groups in biological systems. It also serves multiple functions in a wide variety of metabolisms, such as glycolysis, fatty acid β-oxidation, and biosynthetic pathways utilizing acetyl-CoA [3]. It has been proved that among the known enzymes, about 4% of them require CoA or CoA ester as substrates [4,5]. CoA exists in plants, mammalians and microbial species [6–8]. The lack of CoA in the human body can cause deterioration of the immune system, anxiety, fatigue, impaired sense of balance, depression, irritability, fatigue and aging [9,10]. Nowadays CoA has been applied broadly in clinic to treat atherosclerosis, fatty liver, and so on [11]. On the other hand, very few chemical or biological sensors have been developed to sense CoA. The existing methods to detect CoA are by a reversed-phase high-performance liquid chromatography (HPLC) after the separation and centrifugation from the tissue [12–14]. After purification the sample solutions were injected into the HPLC to detect. The lowest detection limit was 3 pmol [13].

Recently, water-soluble conjugated polymers have got lots of attention as novel optical probes in sensitive biochemical sensors in view of their signal amplification function [15–19]. They have been used to detect nucleic acids, proteins, inorganic ions and biologically small active molecules [20–33]. In this paper, we prepare a new cationic conjugated polymer, that is utilized to optically discriminate CoA among structurally similar biomolecules based on analyte-induced aggregation of the conjugated polymer.

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1 Experimental

1.1 Materials and instrumentations

All reagents were purchased from Aldrich or Alfa-Aesar Chemical Co. and used without further purification. Monomers **1**, **2** and **3** were prepared according to the published literature procedures [34]. ^{1}H NMR spectra were obtained with a Bruker AV400 instrument. UV-vis absorption spectra were taken on a JASCO V-550 spectrophotometer. The gel permeation chromatography (GPC) measurements were performed on Water-410 system against polystyrene standard with THF as eluent. Linear light-scattering spectra were obtained from Hitachi F-4500 spectrofluorometer. Fluorescence measurements were obtained in 3 mL quartz cuvettes at room temperature using Hitachi F-4500 spectrofluorometer equipped with a Xenon lamp excitation source. The excitation wavelength is 361 nm. The water was purified using a Millipore filtration system.

1.2 Synthesis

(1) Synthesis of precursor copolymer (**4**). The mixture of compound **1** (33.6 mg, 0.1 mmol), compound **2** (30.3 mg, 0.1 mmol), compound **3** (148.8 mg, 0.2 mmol) and sodium carbonate (2 mol/L) in 15 mL of THF was degassed, and then $PdCl_2$(dppf) (dppf =1,1'-bis(diphenylphosphine)-ferrocene) was added to the mixture under N_2. And the resulting mixture was stirred at 85°C for 36 h under N_2 atmosphere. After cooling to room temperature (RT), 100 mL of distilled water was added to quench the reaction and the mixture was extracted with chloroform. After the organic solvent was evaporated, the residue was precipitated into MeOH. The crude polymer was precipitated from $CHCl_3$ into MeOH twice and dried under vacuum to give the product as a yellow solid. ^{1}H NMR (DMSO-d_6, 400 MHz): δ_{ppm} = 7.15–7.83 (br), 3.35–3.48 (br), 1.89–2.05 (br), 1.41–1.48 (br), 1.12–1.31 (br), 0.72–0.91 (br). GPC: M_n = 15550, M_w = 70290, PDI = 4.52.

(2) Synthesis of copolymer PFP-PTF. The aqueous trimethylamine (33%) was added to the solution of neutral copolymer 4 in THF by dropwise at RT. After stirring for overnight the solvent was evaporated. The residue solid was precipitated from acetone to give a brown powder. ^{1}H NMR (DMSO-d_6, 400 MHz): δ_{ppm} = 7.45–7.89 (br), 7.18–7.33 (br), 7.06–7.12 (br), 3.58–3.69 (br), 3.40–3.53 (br), 2.95–3.40 (br), 2.11–2.21 (br), 1.95–2.09 (br), 1.45–1.75 (br), 1.17–1.21 (br), 0.75–0.93 (br).

2 Results and discussion

The chemical structure of CoA is shown in Figure 1. Because adenosine triphosphate (ATP), ADP and AMP have similar chemical structures as that of CoA, they are the main interferencing species for CoA detection. Except for the similar adenosine and phosphate moieties as those in ATP, ADP and AMP, the CoA molecule also includes a long side chain that is favorable for hydrophobic interactions. At neutral pH, the phosphate groups of the CoA, ATP, ADP and AMP were deprotonated [10], thus the structure of CoA leads to form a complex with oppositely charged conjugated polymer by cooperative electrostatic and hydrophobic interactions, whereas ATP, ADP and AMP form the complexes with oppositely charged conjugated polymer mainly by electrostatic interactions [25]. Previous studies showed that the cationic conjugated polymers could form tight aggregates in the presence of anionic analytes, where the interchain and intrachain interactions of the conjugated polymer lead to fluorescene quenching [34–37]. Thus, by changing the ion strength of assay solution to screen the electrostatic interactions and remain hydrophobic ones, it is

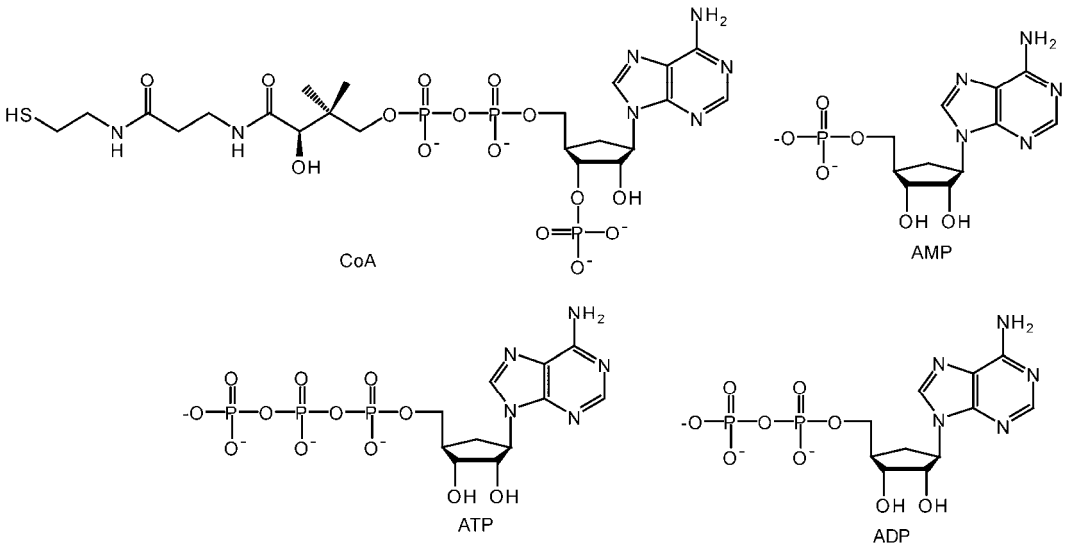

Figure 1 The chemical structures of CoA, AMP, ATP and AMP.

possible to use cationic conjugated polymer to discriminate CoA from ATP, ADP and AMP by fluorescence measurement.

Figure 2 shows the synthetic entry into the water-soluble cationic conjugated polymer (PFP-PTF). The precursor copolymer **4** were prepared by Suzuki's coupling reaction between one equivalent of monomers **1** and **2** with **3** in the presence of 2.0 mol/L Na_2CO_3 in water and $Pd(dppf)Cl_2$ in THF. GPC analyses show number-average molecular weight of **4** is 15550 amu with PDI of 4.52. The polymer **4** was treated with 33% trimethylamine aqueous solution to obtain water-soluble cationic PFP-PTF. The photophysical properties of PFP-PTF were investigated in water. The UV-vis absorption spectra of PFP-PTF exhibit maximum peak at 361 nm, which attributes to the π-π^* transition of the polymer backbone. When we excited the solution at 361 nm, the emission spectra show maximum peak at 475 nm.

Figure 3(a) shows the emission spectra of PFP-PTF ([PFP-PTF]= 4 μmol/L in repeat unit (RU)) with the addition of CoA ([CoA] = 0–6.5 μmol/L) in water with an excitation wavelength of 361 nm. The addition of CoA induces to a obvious quenching of the PFP-PTF emission, and a 87% decrease at 475 nm is obtained. To study the specific interaction of PFP-PTF to CoA, the fluorescence spectra of PFP-PTF in the presence of ATP, ADP and AMP were also investigated under the same condition as CoA. As shown in Figure 3(b), for AMP, a 10% decrease in PFP-PTF intensity at 475 nm is observed, and less than 25% decrease is observed for ADP ([ADP] = [AMP] = 6.5 μmol/L). This showes that ADP and AMP containing less negative charges in comparison to CoA do not induce the aggregation of PFP-PTF by electrostatic interactions. For ATP, an obvious quenching of the PFP-PTF emission at 475 nm is observed, however, the fluorescence quenching efficiency is less than that of CoA in the concentration below 4.0 μmol/L. These results show that the PFP-PTF can discriminate CoA from interferencing ATP ADP AMP species.

Above fluorescence results showed intense interpolymer π-stacking aggregation of PFP-PTF forms in the presence of CoA compared to ATP. Because CoA and ATP have same number of negative charges (four) in neutral water solution, the more efficient fluorescence quenching of PFP-PTF in the presence of CoA should result from the hydrophobic interactions of the side chains of CoA with PFP-PTF except

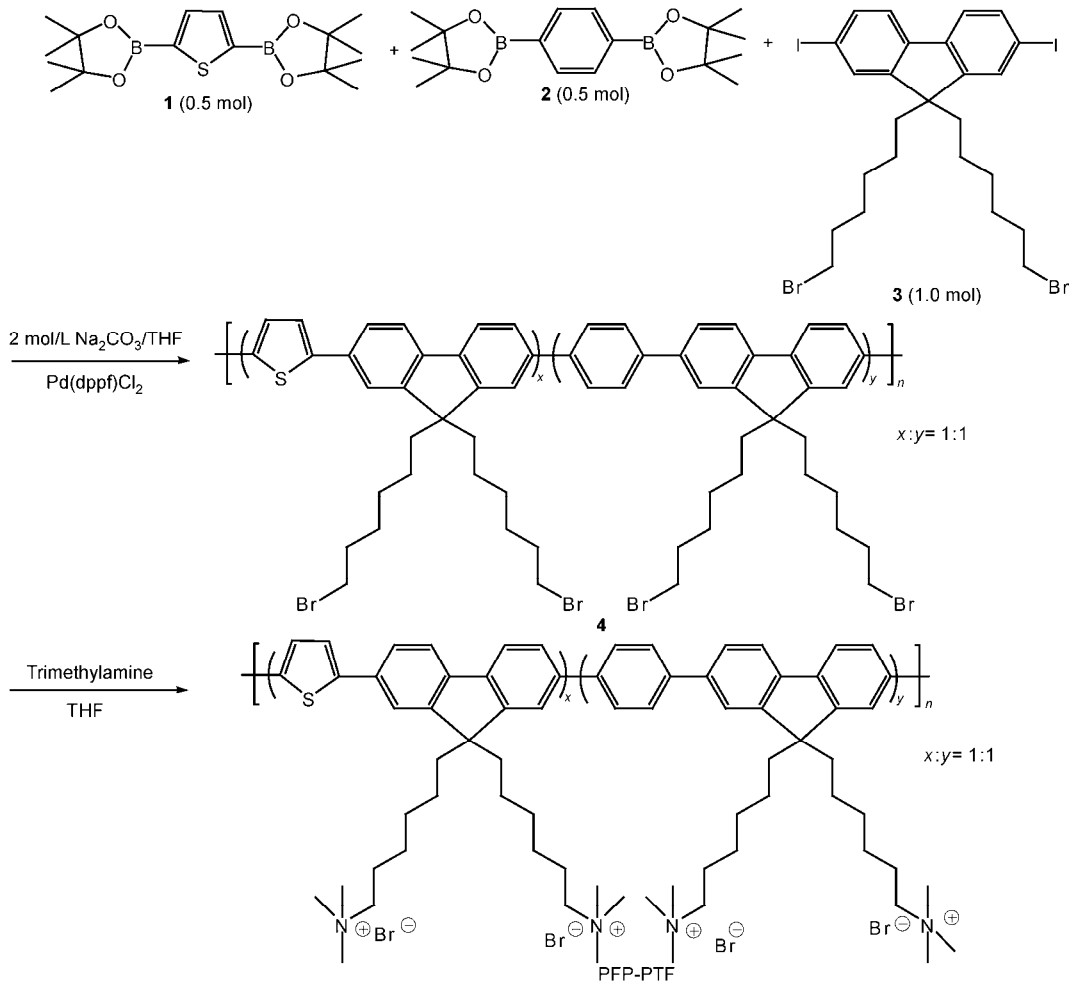

Figure 2 Synthetic routine of polymer PFP-PTF.

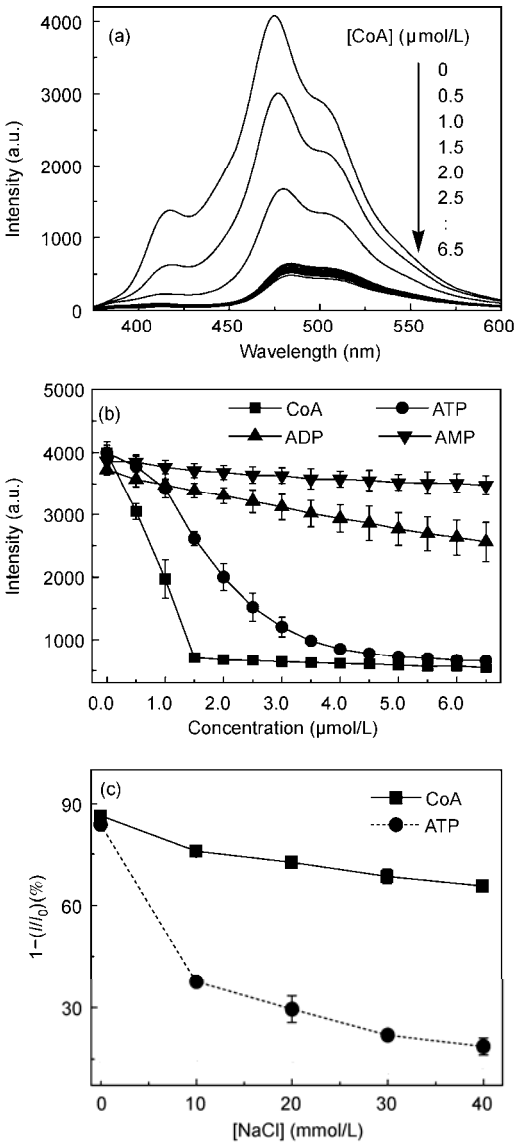

Figure 3 (a) The fluorescence emission spectra of PFP-PTF in H_2O with CoA. (b) The fluorescence intensity of PFP-PTF at 475 nm as function of concentrations of CoA, ATP, ADP and AMP. [PFP-PTF]= 4 μmol/L, [CoA] = [ATP] = [ADP] = [AMP] = 0–6.5 μmol/L. (c) The fluorescence quenching efficiency of PFP-PTF as function of NaCl. [PFP-PTF] = 4 μmol/L, [CoA] = [ATP] = 6.5 μmol/L. [NaCl] = 0–40 mmol/L. The excitation wavelength is 361 nm.

for electrostatic interactions. To give evidence on this quenching mechanism, the dependence of the fluorescence quenching efficiency of PFP-PTF in the presence of CoA and ATP was studied as a function of NaCl concentration in water solution (Figure 3(c)). With the increase of NaCl concentration from 0 to 40 mmol/L, the quenching efficiency alters from 87% to 67% for CoA, and that for ATP changes from 81% to 23%. These results furthermore give evidence that the higher ion strength decreases the electrostatic attractions, and the remaining hydrophobic interactions dominate the formation of PFP-PTF/CoA complex.

Noted that in Figure 3(b), in the higher concentrations of ATP and CoA (>4.0 μmol/L) in water, the similar quenching efficiency of the PFP-PTF emission are observed, where the PFP-PTF can not discriminate CoA from ATP. When the ion concentration of the water solution is inceresed to 40 mmol/L, the quenching efficiency of PFP-PTF by CoA is three times higher than that by ATP (Figure 3(c)), which makes the PFP-PTF possible to sense CoA from interferencing ATP ADP AMP species even in higher concentrations. Thus, the presence of sodium ions in assay solution improves the selectivity of this method.

The aggregation of PFP-PTF in the presence of CoA under 40 mmol/L NaCl is also supported by linear light scattering spectra [38,39], where the electrostatic interactions between them are screened. Figure 4 shows the linear light scattering spectra of PFP-PTF in the presence of CoA, ATP, ADP and AMP in the wavelength range of 200–700 nm. There is no obvious light scattering for PFP-PTF itself. Upon adding CoA, the light scattering intensity of PFP-PTF improves approximately three times in comparison to that of itself, which suggests that the tight aggregation forms in solution in the presence of CoA mainly through hydrophobic interactions. When ATP, ADP and AMP were respectively added, there were not obvious changes of PFP-PTF light scattering intensity because they cannot form compact aggregate with PFP-PTF via electrostatic interactions in this condition. These results are consistent with those of fluorescence measurements. The linear light scattering technique also offers a signal "turn-on" detection of CoA from ATP, ADP and AMP.

3 Conclusions

As compared to the earlier published method to determine the CoA by HPLC [12–14], our detection limit is only 0.5×10^{-6} mol/L, which is much higher than the lowest detection limit of HPLC method was 3 pmol [13], but

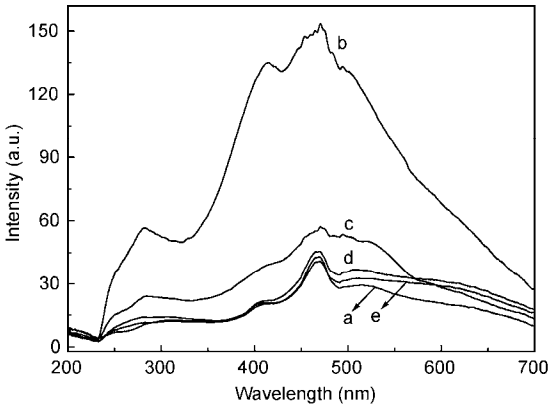

Figure 4 Linear light scattering spectra of PFP-PTF itself (a) and in the presence of (b) CoA, (c) ATP, (d) ADP and (e) AMP in water solution containing 40 mmol/L NaCl. [PFP-PTF]= 4 μmol/L, [CoA] = [ATP] = [ADP] = [AMP] = 6.5 μmol/L. The excitation wavelength is 361 nm.

fluorescence method is more simple and rapid. It makes it possible to directly and real-time detect the concentration of CoA in the tissues sample without extraction and purification.

In summary, a new cationic conjugated polymer was designed and synthesized to optically discriminate CoA among structurally similar biomolecules, ATP, ADP and AMP. The analyte-induced aggregation of the conjugated polymer by π-stacking between their main chains leads to the fluorescence quenching. Except for the similar adenosine and phosphate moieties as those in ATP, ADP and AMP, the CoA molecule also includes a long side chain, which leads to form a complex with oppositely charged conjugated polymer by cooperative electrostatic and hydrophobic interactions. The increase of the ion strength of the assay solution screens the electrostatic attractions, and the remaining hydrophobic interactions dominate the formation of PFP-PTF/CoA complex. At this case, the quenching efficiency of PFP-PTF by CoA is much higher than that by ATP, ADP and AMP, which impart the PFP-PTF to sense CoA from these interference species.

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